Detection of blood volume changes in the rat kidney using an intravascular USPIO contrast agent

P. Storey[1,2], L. Ji[1], L-P. Li[1], P. V. Prasad[1,2]

[1]Evanston Northwestern Healthcare, Evanston, Illinois, United States, [2]Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Introduction: Vasodilation and vasoconstriction are important endogenous mechanisms for maintaining organ homeostasis and function, and the ability of vessels to respond to external stresses and biochemical signals plays a crucial role in organ physiology. Compromised endothelial function is involved in the pathophysiology of diseases such as atherosclerosis, diabetes and hypertension. Monitoring vascular response to mechanical and pharmacological maneuvers therefore provides a tool for detecting and investigating these disease processes. The purpose of this study was to assess the potential of MRI in combination with an intravascular contrast agent to monitor changes in blood volume noninvasively. We used an ultrasmall superparamagnetic iron oxide (USPIO) nanoparticle agent, with a plasma half-life of several hours, and induced systemic vasodilation in rats by intravenous infusion of adenosine. Since adenosine is very short acting, it could be switched on and off repeatedly to confirm reproducibility in each animal. Changes in blood volume were detected by measuring the transverse relaxation rate R2, and the kidney was chosen as a model organ because it is relatively stationary. As a control measure, R2 imaging with and without adenosine was also conducted prior to contrast administration.

Methods: Experiments were performed on 6 male Sprague Dawley rats (267 – 350 g) under an IACUC-approved protocol. Each animal was anesthetized with ketamine (60 – 100mg/kg IP) and Inactin (100mg/kg IP). When unresponsive to footpad pinch, a catheter was inserted in the femoral vein for later administration of adenosine and contrast agent. The animal was then transported to the MRI suite and placed on a cushion at the center of an extremity coil in a right decubitus position. Imaging was performed on a whole-body 3T Twinspeed system (GE Healthcare, Waukesha, WI). A single axial slice was prescribed through one kidney, and images were acquired using a 16-echo Carr-Purcell-Meiboom-Gill (CPMG) sequence with BW = ±62.5kHz, NEX = 4, TR = 1500ms, min TE = 7ms, echo spacing = 7ms, slice thickness = 3mm, FOV = 8 x 4cm, and nominal matrix size = 256 x 192, giving an in-plane resolution of 0.3 x 0.4mm and a scan time of 9min 39sec. Imaging was performed with and without adenosine infusion (500µg/kg/min). Three image sets were acquired prior to contrast administration, with the infusion pump alternately off, on and then off again. A USPIO agent ferumoxytol (Advanced Magnetics Inc, Cambridge MA) was then injected at a dose of 8mg Fe/kg, and the protocol was repeated. Adenosine infusion was switched on and off between alternate acquisitions until a further five image sets had been collected.

Images were analyzed offline using customized routines in Matlab (Natick, MA). R2 values were calculated by fitting the intensity data as a function of TE to a monoexponential decay using a nonlinear Levenberg-Marquardt algorithm. In cases of rapid signal decay, the data were truncated where the intensity fell below about twice its asymptotic value. R2 maps were generated by applying the fitting procedure pixel by pixel, and R2 estimates for the cortex, outer medulla and inner medulla were obtained by fitting the mean intensities in selected regions of interest (ROIs).

Results: Figure 1 shows R2 maps of the kidney in a representative animal for the first 6 acquisitions. The top row (a – c) shows the R2 maps prior to contrast administration, and the bottom row (d – f) shows the corresponding maps after injection of ferumoxytol. From left to right are the results with adenosine infusion off, on then off again. Note that before contrast administration, the map is slightly brighter (R2 is slightly higher) in the renal cortex and medulla with the adenosine infusion on than with it off. Administration of ferumoxytol produces a large increase in R2 throughout the kidney, especially in the inner medulla. It also enhances the R2 response to adenosine. Note that map (f) is marginally darker than map (d), suggesting some washout of contrast agent between the two acquisitions.

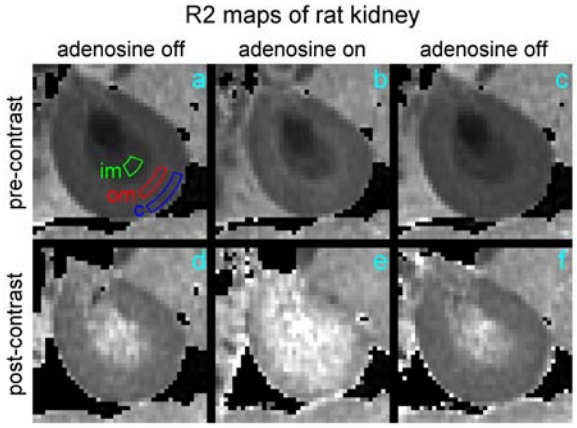

Figure 1: R2 maps of the kidney from a representative rat. Maps (a) – (f) correspond to acquisitions 1 – 6 respectively. Map (a) shows example ROIs in the inner medulla (im), outer medulla (om) and cortex (c). The gray scale used for the maps extends from R2 = $0s^{-1}$ (black) to $60s^{-1}$ (white). Pixels with insufficient signal to perform the fitting procedure have been made black.

The mean R2 values over all the animals are shown in Figure 2. One animal was removed from the scanner after the 5th acquisition due to respiratory distress, so the averages for acquisitions 6 – 8 are evaluated over only 5 animals. As observed in Figure 1, adenosine produces a small but significant increase in the apparent R2 value in all regions of interest prior to contrast administration. Ferumoxytol produces a much larger increase, especially in the inner medulla, and also enhances the response to adenosine. Comparing acquisitions 4, 6 and 8, one observes a slight reduction in R2 over time, suggesting slow washout of the agent. The time constant for the R2 decay as estimated from these three acquisitions is 360±100minutes.

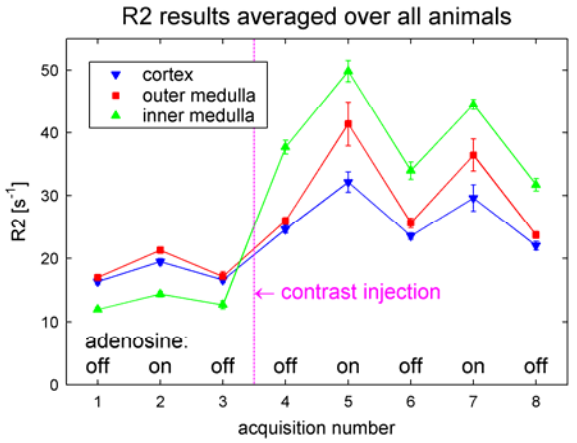

Figure 2: Mean R2 values in the cortex, outer medulla and inner medulla over the course of the experiment. Adenosine infusion was switched on and off between successive acquisitions, both prior to contrast administration (acquisitions 1 – 3) and after contrast administration (acquisitions 4 – 8). Error bars indicate the SEM. The results for series 6 – 8 were averaged over only 5 animals, since one rat was withdrawn from the scanner after series 5.

The table below presents the R2 responses to adenosine (mean ± SEM) in numerical form. The top row shows the responses in the absence of contrast agent, and the second row shows the responses in the presence of ferumoxytol. The difference between the responses is significant in the cortex and outer medulla ($p<0.02$), and highly significant in the inner medulla ($p<0.0001$).

R2 response to adenosine	cortex	outer medulla	inner medulla
Pre-contrast	$3.0 \pm 0.7\ s^{-1}$	$4.2 \pm 0.8\ s^{-1}$	$2.0 \pm 0.6\ s^{-1}$
Post-contrast	$8.3 \pm 1.7\ s^{-1}$	$15.8 \pm 3.7\ s^{-1}$	$13.8 \pm 1.4\ s^{-1}$
p-value for difference	< 0.02	< 0.02	< 0.0001

Discussion: Prior to contrast administration the small apparent R2 responses to adenosine in the renal cortex and medulla may reflect incomplete rephasing of spins on successive echoes due to increased through-plane and in-plane blood flow. While adenosine administration could also affect R2 via alterations in oxygenation levels, one would expect that contribution to be negative, since adenosine increases blood flow without changing oxygen consumption. Similarly, blood volume increases might be expected to make a small but negative contribution to R2 prior to contrast administration, since unenhanced blood has lower R2 than tissue parenchyma.

The large R2 responses to adenosine following contrast administration probably result primarily from blood volume increases, although blood flow changes may make a small contribution. Given the very high relaxivity of ferumoxytol, signal loss due to blood flow is probably minor compared to dephasing by the agent. Note that the contributions of these relaxation processes to the apparent R2 of the tissue are not necessarily additive because of water exchange limitations between the intra- and extravascular spaces. For the same reason it may not be possible to deduce absolute or even relative blood volumes with good accuracy from the present data. We can however conclude that blood volume changes are detectable by MRI using an intravascular USPIO agent.

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